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Short communication

Serological detection of *Toxoplasma gondii* in domestic dogs in the western region of Cuba

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ABSTRACT

We investigated the prevalence of IgG antibodies to *Toxoplasma gondii* in 176 dogs from Havana Province and Mayabeque Province, Cuba, by indirect enzyme-linked immunosorbent assay (iELISA). The overall prevalence was 72.72% (128/176). Dogs living on the cemented floor environment were significantly higher ($p = 0.01$) in being positive for *T. gondii*. The high detection of antibodies to *T. gondii* parasite confirms the outstanding dogs in the West of the Cuban provinces, which is a potential hazard in the region, not only for dogs, but also for public health, considering it is a zoonosis of great importance.

1. Introduction

The protozoan *Toxoplasma gondii* is prevalent in humans and animals worldwide, but little is known about the epidemiology of toxoplasmosis in humans, dogs and other Caribbean countries (Dubey et al., 2016) and in Cuba (Grandía et al., 2013; Rosado García and Medina Fundora, 2014). Although each Caribbean island is separated from the others by the Caribbean Sea and the Atlantic Ocean, tourism, birds migration, and cultural exchange ensure that these islands are visited by people from different regions of the world (Dubey et al., 2016).

The ingestion of contaminated food, like meat with cysts or water with oocysts is the most common way that humans have been infected (Black and Boothroyd, 2000). However, cats excrete environmentally resistant oocysts, and dogs have been linked epidemiologically as risk factor for *T. gondii* infection in humans (Lindsay et al., 1997). This has been related to the fact that dogs are coprophagous, especially with cat feces. Also, oocystes can pass unchanged through the dog's gut, so viable *T. gondii* oocysts have been isolated from feces (Schares et al., 2005). In addition, stray cats and dogs could be used as sentinels of environmental spreading of *T. gondii* oocysts in urban (Alvarado-Esquivel et al., 2014; Munoz and Mayer, 2016) and rural areas (Rosado García and Medina Fundora, 2014), since they are continuously exposed to all infective forms of the parasite (Koch et al., 2016). Thus, the main objective of the present study was to determine the occurrence of anti-*T. gondii* antibodies in dogs from rural and urban areas of the Western region of Cuba.

2. Materials and methods

The field work was conducted in October and December 2013 in the Western region of Cuba (Fig. 1). We tested 176 blood samples of dogs from the municipalities of Habana del Este ($n = 34$), Boyeros ($n = 47$), Cotorro ($n = 45$) in the province of La Habana and San Jose de las Lajas ($n = 50$) of the Mayabeque province. The sampled animals were of different ages, sexes and apparently healthy. An epidemiological adapted questionnaire for each animal was applied to the owners to gather information about their daily routine.

Blood samples were obtained by puncture of the cephalic vein using a 5 ml syringe and then the blood was transferred to sterile containers without anticoagulants. Subsequently, the blood was centrifuged and the sera obtained were packaged in polypropylene bottles and kept at −20 °C until the serological analysis. The sera was then screened for IgG against crude antigen of the *T. gondii* Brazilian strain using the indirect enzyme linked immunosorbent assay (ELISA). In-house ELISAs were adapted from the methodologies of Voller et al. (1976) for canine sera. Microwells (NUNC® Surface Maxisorp) medium binding plates were coated with 100 μl/well of solubilised *T. gondii* antigen at a concentration of 10 μg / ml solubilised in 0.05 M sodium carbonate/bicarbonate

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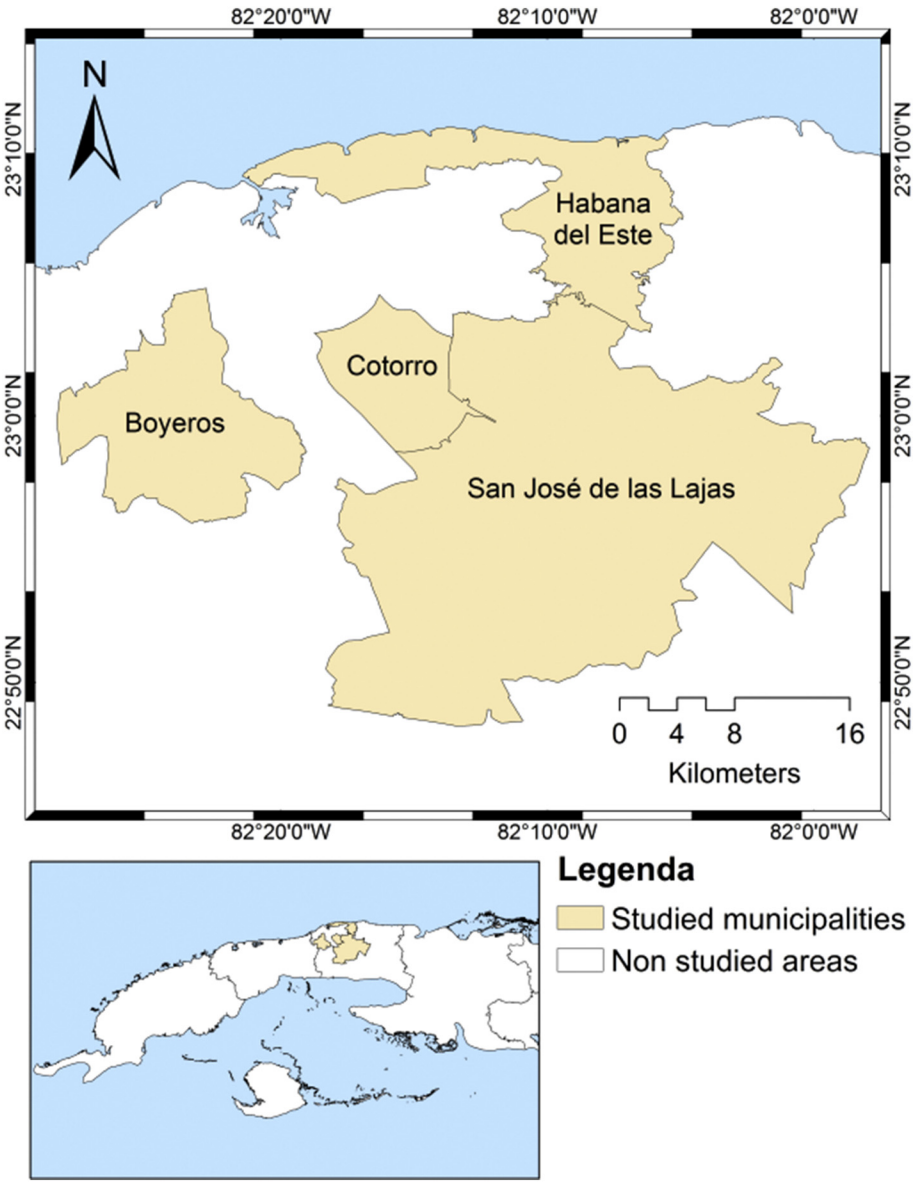

Fig. 1. Cuban regions and provinces showing municipalities of Boyero, San José de las Lajas, Habana del Este and Cotorro, Western region of Cuba.

buffer (pH 9.6) and incubated at 4 °C overnight. Following incubation, plates were washed three times with PBS (pH 7.4) containing 0.05% Tween-20 (PBST). Plates were blocked for 90 min at 37 °C with 200 μl/well of 6% skim powdered milk solution in PBST (Milk/PBST), and washed three times in PBST. Control and test sera were diluted 1:400 in 5% milk/PBST and 100 μl was added to the appropriate microwells. Plates were incubated for 90 min at 37 °C, and washed three times in PBST. Then, 100 μl Anti-Dog IgG (whole molecule) Alkaline Phosphatase (SIGMA®) diluted 1:10,000 in 5% Milk/PBST was added to each well and plates were incubated for 90 min at 37 °C. Plates were washed three times in PBST, and 100 μl (1 mg/ml) substrate *p*-nitrophenyl phosphate (pNPP - SIGMA®) solubilised in diethanolamine buffer (pH 9.8) was added to each well and incubated for 25 min at room temperature. The OD of each plate was measured at 405 nm using a microplate reader. We used 12 negative samples from previously tested animals and a positive control sample of an animal naturally infected from the municipality of Seropedica, state of Rio de Janeiro, Brazil. The cutoff point was calculated using the mean of the negative controls like Frey et al. (1998). To correct the effect of optical density (OD) obtained in each plate, the value of the plates cut line was transformed into 100, so the optical density index was calculated based on the formula DOx100 / "cutoff".

The probable association variables such as breed, gender, age, type of environment, access to the streets and positive cats were performed using the chi-square test with a 95% confidence interval. The simple logistic regression test was used for those variables exhibiting $p < 0.05$ in the chi-square test. For the risk analysis of *T. gondii* infection, the

Table 1
Absolute (n) and relative (%) frequency of positive and negative to reactions of the indirect ELISA test on *Toxoplasma gondii* in 176 domestic dogs from four counties of Western region of Cuba in 2013.

	Habana del leste	Cotorro	Boyeros	San Jose de las Lajas
Positives	22 (64,7%)	35 (77,77%)	32 (68,08%)	39 (78%)
Negatives	12 (35,3%)	10 (22,23%)	15 (31,92%)	11 (22%)
Total	34 (100%)	45 (100%)	47 (100%)	50 (100%)